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DEVELOPMENT OF GENETIC MARKERS FOR PARENTAGE VERIFICATION IN REINDEER

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Development of DNA Markers for Individual Identification and Parentage Verification in Reindeer

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Final Report
prepared for:

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and
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by

Yves Plante

Bova-Can laboratories

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Executive Summary

The Saskatchewan Reindeer Association approached Bova-Can Laboratories to inquire about the availability of genetic diagnostic services for the purpose of animal identification, pedigree verification, and animal registration processes following the guidelines of the Canadian Animal Pedigree Act. With funding from Agri-Food Innovation Fund, a research project was developed and initiated to characterize reindeer DNA markers, assess their informativeness for parentage verification, and develop a set of DNA markers that could be used as routine markers for future fee-for-service parentage analyses for individual breeders.

A reindeer genomic library was constructed and screened for the presence of DNA clones containing short repetitive di-nucleotide sequences called microsatellites. The microsatellites usually show very high genetic polymorphism between individuals, are easy to detect by the polymerase chain reaction (PCR), and their detection and genotyping can be automated. This type of DNA microsatellite marker has become the tool of choice for genotyping and pedigree verification for most livestock species.

Approximately 2000 reindeer DNA clones were screened for the presence of microsatellites using standard published procedures. Of these 86 clones were DNA sequenced and 18 were found to contain large microsatellites. PCR primers flanking the microsatellites were designed and tested on a small panel of reference reindeer DNA samples.

Of the 18 initial reindeer microsatellites, only 3 proved to be useful for routine DNA typing. The remaining 15 markers were either difficult to score, showed low variability, or were actually associated with short interspersed elements (SINEs). The markers show combined probabilities of exclusion ranging from 0.545 for excluding one parent, to 0.897 for excluding both wrongly assigned parents. Because these probabilities are not high enough, other microsatellite markers were assayed in order to obtain probability of exclusion closer to 0.995 or comparable to systems used in other species.

At the present time, we have a panel of 11 microsatellite markers that can be used as routine typing and automated genotyping. It is still too early to estimate the relative probabilities of exclusion, until more reindeer DNA samples are provided for further assays. However, these markers show very high heterozygosity (0.53 – 0.87) indicating that these will be informative and useful for individual identification and pedigree verification.

Introduction

Reindeer and caribou (*Rangifer tarandus*) have a Northern Holarctic distribution and the species represents a significant resource for Northern communities and for the tourism industry. Reindeer are now semi-domesticated in Scandinavia and Russia, and are ranched throughout North America. Although specific markets exist for fresh and processed meat, antlers, and skins; most of the revenues still come from the trade of breeding stocks. With the rapid expansion of this sector of the livestock industry in the recent years, local breeders and their respective breeder associations are now requiring specific parentage testing to verify their pedigrees and to allow for proper registrations under the Canadian Animal Pedigree Act. Recently, local outbreak of chronic wasting disease and the threat of foot and mouth disease pose new challenges to breeders. Identifying good genetic stocks for herd maintenance and replacement will become a new reality. On the other hand, more consumers are now asking about the origin of specific animal products. The European Union is now considering a bio-safety protocol under which all agriculture and agri-food related products will be required to be tagged for traceability. Non compliant may face non-tariff trade barriers. Good traceability programs will help reindeer producers secure their share of the market.

To be competitive, Canadian reindeer breeders must have excellent management practices. One of their goals in maintaining the viability of their stocks is to avoid inbreeding, preserve good genetics, and enhance the general performance of their herds through selective breeding. To meet these breeding goals, the Canadian Animal Pedigree Act, and further product traceability, requires the development of molecular genetic tools.

The availability of genetic markers with relatively high degree of variability has hindered individual identification and pedigree verification services for reindeer breeders. In other livestock species, particularly in cattle, microsatellite DNA typing has become the technology of choice for paternity cases where blood typing results have failed to discriminate the proper sires (Glowatzki-Mullis *et al.* 1995). Although other DNA-based technologies are available, microsatellite

genotype determination and comparison have now been adopted by most Canadian cattle breed association and by all other alternative livestock species for which blood typing reagents are not informative enough or simply not available, as is the case for all cervid species. In general, microsatellites are dinucleotide repeats of the type d(CA)/d(GT)n (where 8<n<50) (Tautz 1989, Weber 1990), but tri-nucleotide and tetra-nucleotide repeats are not uncommon. These microsatellites can be perfect such as d(CA)n, imperfect as d(AC)nTGd(AC)n, or compounded such as d(CA)nTGCd(AT)n (Gibbs *et al.* 1997). Microsatellites make ideal molecular markers because of their usual high genetic polymorphism, simple mode of inheritance and segregation (Panaud *et al.* 1996). Furthermore, microsatellites are fairly easy to detect by the polymerase chain reaction (PCR) procedure, require minimal amount of DNA, and use DNA from various sources. They have found significant applications in genetic mapping (Barendse *et al.* 1994; Bishop *et al.* 1994), genetic mapping of production traits or quantitative trait loci (Plante *et al.* 2001), determination of genetic diversity (McCough *et al.* 1997), and parentage verification in non-traditional livestock species, (Lang *et al.* 1996; Talbot *et al.* 1996).

Although some microsatellite markers may work across species, they are usually less informative (deWoody *et al.* 1995; Talbot *et al.* 1996, Bonnet *et al.* 2002). Few reindeer microsatellites are already available (Wilson et al. 1997; Roed and Midjthjell 1998), and these proved useful for studies of genetic diversity and population structure. To date, no set of reindeer microsatellites have been completely characterized for individual identification and pedigree verification. The general goal of this research project is to develop reindeer specific microsatellites and to incorporate them as part of routine parentage verification testing. The specific objectives are (1) to isolate, clone, and characterize reindeer microsatellites, (2) develop a reliable parentage verification test, (3) offer parentage verification services based on recognized and standardized microsatellite markers, and (4) test the availability of alternate markers from other species as another panel for pedigree analyses.

Material and Methods

Reindeer DNA preparation

Blood samples were collected from 20 unrelated animals (10 females and 10 males) into EDTA coated Vaccutainer™ vials. Reindeer genomic DNA was purified from white blood cells following the standard Proteinase K digestion, phenol-chloroform extractions, and ethanol precipitation method (Plante *et al.* 1992). Reindeer DNA samples were re-suspended in TE buffer (pH 8.0) and kept at 4°C until needed for the DNA library construction and genotyping.

Reindeer genomic DNA library construction

The reindeer DNA library construction and screening followed the procedure outlined by Lang and Plante (1994). For cloning purposes, aliquots (5µg) of DNA from each animal were mixed together. Of this DAN mix, 10 aliquots of 5µg each were digested to completion with the restriction endonuclease *Sau*3A. The restricted DNA fragments where then size-separated though electrophoresis into a 1.5% high strength agarose gels for 60 min. in 1X TBE electrophoresis buffer. Following electrophoresis and Ethidium-bromide staining of the gel, reindeer DNA fragments of the size range 200 – 500 base-pairs (bp) were excised from the gels by electroelution onto a dialysis membrane. These DNA fragments were further purified by organic extraction and ethanol purification. The size-selected reindeer DNA fractions were resuspended in 50µl TE buffer (pH 8.0) and stored at 4°C until needed for plasmid cloning.

The cloning vector BlueScript pSK- (Stratagene) was linearized with the restriction enzyme *Bam*HI and dephosphorylated with Calf Intestinal Alkaline Phosphatase (CIAP) following the manufacturer's (GIBCO BRL Life Technologies) recommendations. Reindeer DNA fragments were ligated into the dephosphorylated pSK- vector using molar ratios of 1:1, 1:5, and 5:1 in 10µl reaction volumes at 4°C for 16 hrs. Competent *E. coli* cells (DH5α) were tranformed with 2µl (approximately 30 – 50ng) of each ligation reaction mixes and selected onto 2YT agar media containing 50µg/ml of ampicillin, and coated

with 2% X-Gal and IPTG. The agar plates were incubated inverted at 37°C for 16 hrs.

A similar reindeer library construction approach was devised to pre-select for the enrichment of DNA sequences containing microsatellites prior to cloning, following the procedure of of Armour *et al.* (1994) and Gibbs *et al.* (1997). The procedure is based on the hybridization of reindeer DNA fragments to synthetic dinucleotide repeated elements fixed onto solid supports. Following hybridization, the reindeer DNA population enriched for microsatellites is recovered, and this population is subjected to classical cloning and screening protocols. Although a large effort was used to prepare several enriched reindeer microsatellite libraries (Prosofsky, 2000), we did not find a significant number of informative clones after initial screening.

Reindeer DNA library screening

Bacterial colonies were transferred from the agar plates onto HybondN+ nylon membranes (Amersham-Pharmacia). The cells were lysed in 10% SDS for 3 min., denatured in 0.5M NaOH, 1.5M NaCl for 5 min, and neutralized in 0.5M Tris-HCl pH 8.0, 1.5M NaCl for 5 min. These bacterial colony lifts were finally washed in 2X SSC. The membranes were air-dried and the plasmids were fixed to thse membranes by baking at 80°C for 2hrs. These colony lifts were kept in sealed plastic bags at room temperature until needed fro the reindeer microsatellite screening procedure.

The colony lifts were wetted in 5X SSC and pre-incubated in 35ml of hybridization solution (0.262M Na_2HPO_4, 7% SDS, 1% BSA, and 10mM EDTA) for several hours at 65°C with gentle shaking. A microsatellite DNA probe was prepared by labeling a synthetic poly d(AC)/d(GT) oligonucleotide (Amersham-Pharmacia) with αP^{32}dCTP, random hexamers, and Klenow DNA polymerase. This microsatellite DNA probe was denatured and added to the colony lifts with 15ml of fresh hybridization solution, incubated under the same conditions. The membranes were then washed several times in 0.5X SSC, 0.1% SDS at 65°C to remove unbound probe and unspecific signals. The colony lifts were air-dried,

marked with specific keyholes, wrapped in plastic film, and exposed to X-Ray films. Positive signals were aligned to the respective agar plates, and corresponding colonies were picked and grow in 3ml of 2YT liquid media containing 50µg/ml ampicillin. Plasmid DNA clones positive for reindeer microsatellites were recovered following the standard alkaline lysis procedure (Sambrook *et al.* 1989) with the addition of an extra ether extraction and ethanol purification. Aliquots of each plasmid (2µg) were prepared for sequencing.

Reindeer microsatellite sequencing and PCR primer design

Plasmid containing the reindeer DNA inserts were sequenced using the Amersham-Pharmacia T7 Sequencing kit with both T3 an T7 sequencing primers. Sequences were resolved into 5% poly-acrylamide gels and exposure to X-ray films. Reindeer DNA sequences were read by independently by three persons and ambiguous sequences were repeated from fresh plasmid DNA preparations. Only reindeer DNA sequences containing large microsatellites, of the type d(AC)/d(GT) > 8, or from imperfect repeats, were further considered for characterization. PCR primer pairs flanking each microsatellite were designed with the help of the software Primer Design© and synthesized (Alpha DNA Inc.). each primer pairs was optimized for annealing temperature, and Mg++ concentration.

For optimization of each reindeer microsatellite marker, the PCR reaction contained approximately 50ng of reindeer DNA, 20pmoles each primer,1X PCR buffer (Sigma, St-Louis), 1.0 mM to 3.5 $MgCl_2$ (0.5mM increments), 100umoles dNTPs, and 0.5U *Taq* DNA Polymerase (Sigma, St-Louis) in a 25ul total reaction volumes. The PCR amplification cycle parameters will be a denaturation step at 94 °C for 30 sec, annealing step at 50 °C to 68 °C (2°C increments), an extension step at 72 °C for 30 sec, for 30 cycles, with a final extension step at 72 °C for 10 min. to ensure complete polymerization. The PCR reactions will be loaded onto 2% agarose gels for electrophoresis. The quality of the PCR reactions (intensity of the PCR DNA fragments, sharpness, and the number of amplified DNA bands)

determined the proper PCR conditions to use when genotyping a panel of 20 unrelated reindeers.

Only PCR primer pairs giving a unique and clear amplification product, on an agarose gel were considered further for population screening. Optimization and initial scoring were performed independently by two persons.

Reindeer microsatellite marker statistics

Each reindeer microsatellite marker was tested on a panel of 20 unrelated animals. Relevant statistics included the number of alleles and their respective size (in nucleotides), the heterozygosity (Nei and Roychoudhury 1974), the polymorphism information content (PIC) (Botstein *et al.* 1980), and the probabilities of exclusion (Jamieson and Taylor 1997). The overall probability of exclusion was also estimated for the most informative set of reindeer microsatellites.

Results and Discussion

Initial screening of the reindeer microsatellite libraries targeted approximately 2000 clones. Of these clones, 86 clones contained reindeer microsatellites. Further sequence analyses revealed that only 18 of these 86 potential microsatellites contained enough flanking sequence information surrounding the reindeer dinucleotide repeats to be used as targets for the design of PCR amplification primer pairs. Finally of these, only 3 reindeer microsatellites markers could be reliably used for genotyping. The other microsatellites proved to be associated with short interspersed elements or SINEs (Buchanan *et al.* 1999), or were monomorphic in the panel of reindeer reference samples. In both cases, these reindeer microsatellite markers were not informative for individual identification and parentage verification and these were not considered any further. However, these markers have already proved their informativeness and usefulness for species and populations evolutionary studies as shown by the work of Buchanan et al. (1999). In any case, the sequences for the monomorphic reindeer microsatellite markers will be shared with those

involved in cervid population genetic studies. The Cervid Standing Committee of the Internal Society for Animal Genetics (www.isag.org) is currently building a resource webpage that will deposit all available sequence information related to microsatellite in all cervid species. The author of this report is the contact person for the Committee and responsible for the collection of marker sequence information and relevant statistics related to cervid microsatellite markers.

Table 1 lists the 3 reindeer microsatellite markers with their associated statistics. Reindeer microsatellite SRC-RT-22 is the most informative markers with 9 alleles detected and an expected heterozygosity of 0.83. This marker also showed the best probability of exclusion of 0.655 for the sire when the dam is known. The same marker has a power of 0.482 in excluding one parent when the other parent is unknown, and an exclusion probability of 0.832 for both parents when their genotypes are known. Markers SRC-RT-6 and SRC-RT-45 show intermediate values of heterozygosity and relative power of exclusion. Unfortunately these 3 reindeer microsatellites are not as informative as expected and as those previously described by Wilson et al. (1997) and Roed and Midtjjell (1998). However the reindeer described these later studies are posing problems of their own in terms of published information, sequences, and informativeness (K. Roed, personal communication, Cervid Standing Committee, ISAG meeting 2002, Gottingen, Germany).

Given the three reindeer microsatellite markers described here, the overall probabilities of exclusion is 0.897 for rejecting both parents, is 0.743 for excluding the sire when the dam is known, and is 0.545 for excluding one parent when the other parent in unknown (Jamieson and Taylor 1997). Similar to the expected heterozygosity, these probabilities are somewhat lower then those expected by the two previously cited studies (albeit that these studies are showing some inconsistencies) but still better then those presented by DeWoody *et al.* (1995).

Table 1: Informative reindeer microsatellites fully characterized in this study.

Marker	Repeat Sequence	Primer Sequences
SRC-RT-6	$(AC)_{20}$	Forward GCA TGT GGA AGC CTG TGG TA Reverse GAA GGA AGC AGA GCT GAG GA
SRC-RT-22	$(AC)_3T(AC)_{40}$	Forward GCA ATA GGC TTT ATG GCT CT Reverse CAT CTT CTT CCA TCC ACT GC
SRC-RT-45	$(AC)_{19}$	Forward CTG AGG CAA ATG AAC AAA CG Reverse CTA CAG TAT TC[illegible] GGC CTG GA

Marker No.	Alleles	H[1]	PIC[2]	Exclusion Probabilities[3]		
				Sire	One	Both
SRC-RT-6	2	0.44	0.35	0.173	0.099	0.263
SRC-RT-22	9	0.83	0.80	0.655	0.482	0.832
SRC-RT-45	2	0.22	0.19	0.097	0.024	0.167
Combined Probability of Exclusion				***0.743***	***0.545***	***0.897***

[1] Nei and Roychoudhury (1974), [2] Botstein *et al.* (1980), [3] Jamieson and Taylor (1997)

Observing lower than expected heterozygosity for the reindeer microsatellites described in this study is surprising in this particular species since it is thought of as one of the most genetically diverse cervid species. Our reference panel of 20 individuals is very limited, all samples came from the same breeder, and it is likely that inbreeding in this reference herd caused a loss of genetic variability and several possible alleles went undetected. New samples were requested (in 2000, 2001, and then again in 2002) from the Saskatchewan Reindeer Breeder Association. To date no samples were forwarded to the laboratory. However, this winter the Saskatchewan Reindeer Breeder Association will be able to provide 40 new samples from individuals collected in Alaska. These new samples will allow us to test these reindeer markers on a larger panel of reindeer reference samples. It is anticipated that an increased number of alleles will be detected at markers SRC-RT-6 and SRC-RT-45. Indeed

microsatellites with dinucleotide repeats in the range of 20 have proved to be extremely polymorphic in other species (Lang *et al.* 1996; Talbot *et al.* 1996; Slate *et al.* 1998).

For individual identification and parentage verification, it is evident that several more markers are necessary to attain the high probabilities of exclusion necessary to gain confidence in power of the tests. Two other published studies have described reindeer microsatellite sequences deemed informative for population studies. Wilson *et al.* (1997) described 13 caribou microsatellites. According to these authors, 12 of these markers are informative with heterozygosities ranging from 0.555 to 0.867. This high level of polymorphism is ideal for these markers to be considered as informative for pedigree verification. Using the information from Wilson *et al.* (1997), we have tested these 12 markers and have found that 5 did not produce reliable results or failed PCR amplification. Problems associated with these markers have recently been confirmed by other groups (D. Paetkau, Wildlife Genetics International, personal communication, and K. Roed, Norwegian College of Veterinary Medicine, personal communication). Furthermore, two of Wilson et al. (1997) markers are from the same locus, and two more mapped to the same chromosome, making these markers very limited in their usefulness to resolve pedigree problems.

However, we were able to compile more information regarding some of these reindeer markers, and also use markers from other species and produce a set of 11 markers. These markers have been designed for use in an automated genotyping systems and are described in Table 2. These markers have only been tested in a limited number of animals, but we were able to detect from 6 to 10 alleles at each of these. Estimates of heterozygosity for these markers range from 0.53 to 0.87 (D. Paetkau, personal communication), which indicate that these markers will be informative for individual identification and pedigree verification. As Institutional members of ISAG, we will recommend that these be adopted for routine testing and for international comparisons testing for the purpose of individual identification and pedigree verification.

Table 2: First panel of microsatellite markers for use in automated genotyping system for reindeer pedigree verification.

NAME	SEQUENCE	LABEL	SIZE RANGE	ALLELE#
RT13	CAT CCC AGA ACA GGA GTG AG AGA GAA TGG CCC AGT GTT AG	6-FAM	296-312	8
RT24	GTG TAT CCA TCT GGA AGA TTT CAG CAG TTT AAC CAG TCC TCT GTG	TET	207-235	8
RT1	AGA CCC ATC TTC CCA TCC TCT T TGC CTT CTT TCA TCC AAC AA	HEX	230-250	7
BL42	ACA AGT CAA GGT CAA GTC CAA ATG CC GCA TTT TTG TGT TAA TTT CAT GC	6-FAM	240-260	8
OhN	GCA ACC AAT AGG ATA GGT CG GCT GGA TGG AAC TGA AAG TC	TET	250-296	7
BM6506	GTG GTA AAG AGA TGG CAT AGC A AAC TTA GCA ACT TGA GCA TGG	HEX	183-223	8
RT7	ACT TTT CAC GGG CAC TGG TT CCT GTT CTA CTC TTC TTC TC	6-FAM	217-231	6
BM3507	GCC CAA AGA AAG AAG TAT GTG C GTA GTG CGG AGT CAG TCA TGT G	TET	164-176	5
BM4513	TCA GCA ATT CAG TAC ATC ACC C GCG CAA GTT TCC TCA TGC	HEX	113-139	10
BM888	AGG CCA TAT AGG AGG CAA GCT T CTC GGT CAG CTC AAA ACG AG	6-FAM	170-192	7
RT5	CAG CAT AAT TCT GAC AAG TG GTT GAG GGG ACT CGA CTG	TET	94-118	8

Two of these markers may require further analyses, BM888 and OhN. These two markers are very informative, having at least 7 different alleles. BM 888 posses an unusually large allele of 264 nt, and OhN shows a series of unusual allele sizes for a tetra-nucleotide repeats. These two markers may create some confusion and it is only with further work that one will become confident in accurately scoring these two markers or to decide to replace them.

As with any set of markers, in any given species, some individual identification or parentage verification cases will not be solved by using a single panel of markers. The recent work of Bonnet *et al.* (2002) presents data on the analysis of 38 different microsatellites tested on 16 different cervid species, three of those were shown to be informative in reindeer and merit further investigations. Finally, Roed and Midthjell (1998) described 17 reindeer microsatellites, three of which were successfully tested in our laboratory, and three more are still under investigation. We are confident that the markers described in Table 2 will produce probabilities of exclusion of the order of 0.95 to 0.995, similar to other systems currently used in other livestock species.

Conclusion

This research project fully characterized 17 microsatellite markers for use in individual identification and pedigree verification in reindeer. We now have a panel of 11 markers that can be incorporated in simple multiplex PCR reactions allowing for rapid and inexpensive testing using automated genotyping systems. This panel will be added to our basic typing services for reindeer breeders and their respective associations.

At the present time, we are encouraging reindeer breeders to submit samples to further characterize this particular panel of markers. We have determined that all markers are easy to detect and show large number of alleles. However, we still have to compute proper probabilities of exclusion using a large number of reindeer DNA reference samples. Continuous communication with the Saskatchewan Reindeer Association will facilitate further characterization of alternate microsatellite markers that may be required to define alternate markers to solve difficult cases.

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References

Barendse W., Armitage S.M., Kossarek L.M. *et al.* 1994. A genetic linkage map of the bovine genome. *Nature Genetics* 6: 227-235.

Bishop M.D., Kappes S.M., Keele J.W. *et al.* 1994. A genetic linkage map for cattle. *Genetics* 136: 619-639.

Bonnet A., Thevenon S., Maudet F., and Maillard J.C. 2002. Efficiency of semi-automated fluorescent multiplex PCR with 11 microsatellite markers for genetic studies of deer populations. *Animal Genetics* 33: 343-350.

Botstein D., White R.L., Skolnick M., and Davis R.W. 1980. Construction of a genetic linkage map in human using restriction fragment length polymorphisms. *American Journal of Human Genetics* 32: 314-331.

Buchanan F., Crawford A., Strobeck C. *et al.* 1999. Evolutionary applications of MIRs and SINEs. *Animal Genetics* 30: 47-50.

De Woody J.A., Honeycutt R.L., and Skow L.C. 1997. Microsatellite markers in white-tailed deer. *The Journal of Heredity.* 86: 317-319.

Gibbs M., Dawson D.A. Mc Calmey C. *et al.* 1997. Chicken microsatellites markers isolated from libraries enriched for simple tandem repeats. *Animal Genetics* 28: 401-417.

Glowatzki-Mullis M.L., Gaillard C., Wigger G. and Fries R. 1995. Microsatellite-based parentage control in cattle. *Animal Genetics* 26:7-12.

Jamieson A., and Taylor C.S. 1997 Comparison of three probability formulae for parentage exclusion. *Animal Genetics* 28:397-400.

Lang K.D.M. and Plante Y. 1994. Fifteen polymorphic bovine dinucleotide microsatellites. *Animal Genetics* 25:373.

Lang K.D.M., Wang Y. and Plante Y. 1996. Fifteen polymorphic dinucleotide microsatellites in llamas and alpacas. *Animal Genetics* 27:373.

McCough S.R., Chen X., Panaud O. *et al.* 1997. Microsatellite marker development, mapping and applications in rice genetics and breeding. *Plant Molecular Biology* 35: 89-99.

Nadesalingham J., Plante Y., and Gibson J.P. 2001. Detection of QTL for milk production on chromosome 1 and 6 of Holstein cattle. *Mammalian Genome* 12: 27-31.

Nei M. and Roychoudhury A.K. 1974. Sampling variances of heterozygosity and genetic distances. *Genetics* 76: 379-390.

Panaud O., Chen X., and McCough S.R. 1996. development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa L.*). *Molecular and General Genetics* 252: 597-607.

Plante Y. Gibson JP, Nadesalingam, J, Mehrabani-Yeganeh H., *et al.* 2001. Detection of quantitative trait loci affecting milk production traits on 10 chromosomes in Holstein cattle. *Journal of Dairy Science* 84: 1516-1524.

Plante Y., Schmutz S.M., Lang K.D.M. and Moker J.S. 1992. Detection of leucochimerism in bovine twins by DNA fingerprinting. *Animal Genetics* 23: 295-302.

Prosofsky B.J. 2000. Development of DNA microsatellite markers for parentage verification for bison (*Bison bison*) and reindeer (*Rangifer tarandus*). B.Sc. Thesis, University of Saskatchewan.

Roed K.H. and Midthjell L. 1998. Microsatellites in reindeer, rangifer tarandus, and their use in other cervids. *Molecular Ecology*. 7: 1173-1176.

Slate J., Coltman D.W., Goodman S.J. *et al.* 1998. Bovine microsatellite loci are highly conserved in red deer (Cervus elaphus), sika deer (Cervus Nippon) and Soay sheep (Ovis aries). *Animal Genetics* 29: 307-315.

Sambrook J., Fritsch E.F., and Maniatis T. 1989. Molecular Cloning – A laboratory manual. Cold Springs Harbor Laboratory Press, New York, USA.

Talbot J., Haigh J., and Plante Y. 1996. A parentage evaluation test in North American elk (Wapiti) using microsatellites of ovine and bovine origin. *Animal Genetics 27*: 117-119.

Tautz D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA. *Nucleic Acids Research* 12: 4127-4138.

Weber J.L. 1990. Informativeness of human (dC-dA)n-(dG-dT)n polymorphism. *Genomics* 7: 524-553.

Wilson G.A., Strobeck C., Wu L., and Coffin J.W. 1997. Characterization of microsatellite loci in caribou *Rangifer tarandus*, and their use in other artiodactyls. *Molecular Ecology*. 6: 6977-6999.